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REPORT OF THE MINISTER (EALTH'S SPECIAL PANEL
ON FRANCISELLA RESEARCH AT THE UNIVERSITY OF VICTORIA

VICTORIA -- Health Minister Peter Dueck released today findings of tne 10-member Panel on Francisella Research at University of Victoria.

The independent panel was formed August, 1988, as result of public concern over the safety of anticipated microbiological research using Francisella organisms at the University of Victoria.

The panel described the current proposal of the principal researcher Dr. Francis Nano as "the relatively minor genetic manipulation of a non-hazardous organism."

"I am confident that the panel has thoroughly addressed the safety concerns surrounding the research activities involving Francisella organisms at the university," said Dueck.

"I am also pleased to announce the panel has found that the public's health and safety are being protected and maintained. The findings and recommendations of the panel will help restore confidence in the university's research endeavours," he added.

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The report of the panel stated the Francisella novicida organism is not considered hazardous to man and that the currently proposed research can be conducted safely in the present Level Two Biohazard facility at the university.

The panel, chaired by Chief Provincial Health Officer, Dr. Hugh Richards, added that the research does not involve the actual infection of laboratory animals.

Copies of the Report of The Minister of Health's Special Panel on Francisella Research at the University of Victoria will be made available.

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OCT 6 - 1988

REPORT OF

THE MINISTER OF HEALTH'S

SPECIAL PANEL

ON FRANCISELLA RESEARCH

AT THE

UNIVERSITY OF VICTORIA

September 26, 1988

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REPORT OF THE MINISTER OF HEALTH'S SPECIAL PANEL ON FRANCISELLA RESEARCH AT THE UNIVERSITY OF VICTORIA

INTRODUCTION

The Minister of Health's Special Panel on Francisella Research at the University of Victoria was formed as a result of public controversy and concern in the summer of 1988, over the safety of anticipated microbiological research using Francisella organisms at the University of Victoria. No such research had started, nor had these organisms been brought into the community, and the President of the University had given a public undertaking that such would not take place until all health and safety issues had been adequately addressed.

The principal researcher, Dr. Francis Nano, had applied for and later cancelled plans to obtain research funding from U.S. military sources. He also submitted a research proposal to the British Columbia Health Care Research Foundation, for which he was successful in obtaining funding approval. Part of this funding has been withheld pending the outcome of the Special Panel's report to the Minister. The application to Agriculture Canada for a permit to import the Francisella organisms was granted for the importation of Francisella novicida only, an organism considered non-pathogenic for man. It was the decision of the permitting authority that the laboratory facilities at the University required upgrading before the importation of other species of the Francisella organism could be allowed.

Public concern was aroused with a report on the proposed research by the Deputy Medical Health Officer of the Capital Regional District to the Board of the Capital Regional District concerning public health and safety issues.

At this stage, the Minister of Health for British Columbia, with the full cooperation of the administration of the University of Victoria and the Capital Regional District, on August 29, 1988, announced the establishment of an independent panel of experts to review the situation:

1. to ensure that the public's health and safety was protected, and

2. to restore the confidence of the public in research activities at the University of Victoria.

SCOPE OF CURRENT RESEARCH PROPOSAL

Dr. Nano specified for the Panel the scope of the present research proposal, from which the following direct quotes are taken:

"1. Development of Genetic Tools

"One of the advantages of using F. tularensis to study intracellular growth is that one should be able to study mutant phenotypes that would be lethal in an obligate intracellular parasite. In order to efficiently create and characterize such

mutants, one needs to be able to use a variety of genetic tools. The important tools for this study are the ability to introduce DNA into F. tularensis and the ability to mutagenize with transposons in order to genetically "tag" relevant loci. Since transformation with chromosomal DNA has already been demonstrated in F. novicida using F. tularensis DNA (9), the main challenge for us would be to perfect this technology with F. tularensis. The use of electroporation may allow us to achieve high transformation frequencies without extensive testing of biochemical methods. In order to have DNA in trans in F. tularensis, we would need to have a plasmid vector that is able to replicate in that host. A number of broad host range cloning vectors (incompatibilities P and W) have been developed (16), and one of these will undoubtedly replicate and express a selectable marker in F. tularensis. Broad host range cosmids (17) could also be introduced via a tri-parental mating system (18). There is no plasmid present in the LVS strain, but a plasmid has recently been discovered (26) in a related strain, and this plasmid could be the basis of a shuttle vector.

Transposon mutagenesis will be attempted using broad host range "suicide vectors" (19) introduced by mating, or by the transformation of DNA carrying transposons. Three transposons will be used in initial studies: Tn5 (21), since it usually transposes in a random fashion; TnPhoA (20), since it can help identify outer membrane protein; and mini Tn10Km (22), since it jumps at a high frequency and is stable once inserted.

Molecular cloning and expression of F. tularensis genes in E. coli will be an essential component of our studies. I anticipate this not to be a problem since I have already isolated recombinant clones (cosmid and pUC recombinants) that express five major polypeptides in the outer membrane.

2. Biochemical Analysis of the Outer Membrane of F. tularensis

The interaction of bacteria and their environment is largely through bacterial surface constituents and secreted products; and in gram-negative pathogenic bacteria, a role for outer membrane proteins in pathogenicity has been demonstrated several times. Although the surface of F. tularensis is probably largely covered by the capsule layer, it is likely that outer membrane proteins play a role in cell and tissue invasiveness (F. novicida reportedly lacks a capsule but causes a similar disease with similar lesions in the mouse). In our study of the LVS strain, we will ask basic questions about the constituents of the outer membrane. Which proteins are the outer membrane proteins? Which proteins play a structural role, porin role or a role in bacterium-host cell interaction? An understanding of the basic components of the F. tularensis outer membrane will provide a

foundation for studying outer membrane components from strains of different virulence.

To demonstrate that a putative outer membrane protein is indeed localized in the outer membrane requires multiple criteria. Past investigators have used the susceptibility of proteins on intact cell to proteinase digestion, iodination, and reaction with antibody as evidence of surface exposure. Co-purification with known outer membrane constituents, such as lipopolysaccharide, is also a criteria for localization to the outer membrane.

Sandstrom et al (11) have called a Sarkosyl insoluble extract of F. tularensis LVS an outer membrane preparation on the basis that the four proteins found in this fraction stimulate T-helpers cells from vaccinated individuals, and that two of the proteins can be surface labeled with 125_I. We will extend the work of Sandstrom et al by fractionating membranes by sucrose gradient centrifugation. Identity of the fractions will be established by examining the content of known inner and outer membrane components such as lipopolysaccharide, cytochromes, and succinate dehydrogenase. Suspected outer membranes will also be examined for their susceptibilities to proteinase K digestion on capsule proficient and deficient strains.

The recombinant clones that I have isolated which produce putative outer membrane proteins may serve as useful reagents. Localization of these proteins in E. coli's outer membrane would strongly suggest that they are outer membrane proteins. The recombinants should also allow us to prepare monospecific polyclonal antibody antibody by reacting the clones with serum and then .... with the surface of F. tularensis."

(Taken from the Application for B.C. Health Care Research Foundation Research Funding #15 (88-1))."

Dr. Nano qualified the above with his 6 to 12 month planned program of genetic experiments using F. novicida only, described in a further document he provided to the Panel, from which the following direct quotes are taken:

"1. *Generate recombinant clone bank of Francisella novicida DNA in plasmid vectors. This will all be done in an Escherichia coli EK1 system, i.e., an approved safe E. coli strain.*

2. *Screen recombinant clone bank with polyclonal antibody made against whole, formalin fixed F. novicida (antibody previously prepared at Rocky Mountain Laboratories, NIH).*

3. *Analyze putative positive recombinants by Western Immunoblots.*

4. *Inactivate expression of recombinant product by transposon insertion. Verify lack of expression by Western blots."*

The Panel characterized the above technical descriptions in summary as the relatively minor genetic manipulation of a non-hazardous organism, utilizing techniques which do not involve the actual infection of laboratory animals.

CONCERNS OF THE LOCAL HEALTH AUTHORITY

Dr. Timothy Johnstone, Deputy Medical Health Officer for the Capital Regional District, provided the Panel with a list of concerns, which is quoted in full as follows:

"1. *Are there negative health impacts likely with genetic manipulation of (a) Francisella novicida; (b) Francisella tularensis, LVS and B38 strains in respect of (a) the local animal population, or (b) the local human population on Vancouver Island?*

2. *Is a secure level 3 containment laboratory necessary for the Francisella research, as recommended by the Chief Biosafety Officer of Health and Welfare Canada?*

3. *What measures will be necessary to protect the health of staff and students in the University of Victoria laboratories involved in Francisella research?*

4. *Will immunization of staff and students be needed with the U.S. Army vaccine already administered to Dr. F. Nano, which is not licensed by the U.S. Food and Drug Administration or the Canadian Bureau of Biologics for civilian use?*

5. *What measures will be necessary to dispose of liquid and solid waste materials, including animal carcasses, from the Francisella experiments? Should liquid waste be prohibited from entry to the sanitary sewer system? What disinfection procedures will be used? Where will incineration take place? Will B.C. Ferries be involved in transportation of pathological waste? If so, what precautions will be taken?*

6. *Should the fact that Victoria is in an active earthquake zone be a consideration in respect of possible siting of the Francisella laboratory?*

7. *What emergency response protocols should be in place before construction of such a laboratory, and who will be responsible for co-ordination of emergency response procedures and exercises?*

8. *In the event that a Francisella laboratory is constructed, what measures will be taken in relation to security, since local activist groups plan to break into such a laboratory, and release the experimental animals?"*

Dr. Johnstone emphasized that the Capital Regional District is not opposed to academic research, and that it seeks a cooperative working relationship between the health authority and the University to ensure public health and safety.

PERMIT PROCESS FOR THE IMPORTATION OF HUMAN AND/OR ANIMAL PATHOGENS INTO CANADA

The responsibility for this activity lies with Agriculture Canada, which works in cooperation with the Laboratory Centre for Disease Control of Health and Welfare Canada, in reviewing applications. At present, a permit has been issued to Dr. Nano for the importation of Francisella novicida only, for research within the present facilities at the University of Victoria.

The Panel was advised that legislation pertaining to this process is under review, with improvements in and clarification of procedures expected within the next year or so.

THE SIGNIFICANCE OF DIFFERENT STRAINS OF FRANCISELLA ORGANISM

The Francisella genus of organisms consists of a number of species and strains, each of which differ in their potential to cause disease in humans and animals.

The present research proposal only involves the use of the F. novicida species which is considered non-pathogenic to man. Any change to a more virulent form through genetic manipulation would include the infection of live animals, a procedure which is not involved in the current research project.

Within the species Francisella tularensis, there are a number of strains with varying disease-causing potential. The low virulence strains include the vaccine (LVS) and the B38 strains. Other strains range in virulence from low to high. The present research proposal does not involve any F. tularensis strains, however, work with this organism may be contemplated in the future.

CONCLUSIONS AND RECOMMENDATIONS

The Panel understands that both the University and local health authority recognize the need for a positive working relationship in the Biohazards Committee. This would ensure a full exchange of pertinent information and the continuing development of protocols and protective measures appropriate to the research being contemplated prior to commencement, with adequate time to train and protect all those associated with the research activity, to include students, researchers and maintenance staff.

The Panel recognized the importance for all concerned and for the public's reassurance, that a clear understanding exists of each participant's role on the Biohazards Committee, and that there be a means for effective communication within the University and between the University and appropriate outside agencies to ensure a proper auditing of the research activity.

The Biohazards Committee and staff should remain current in the matter of biohazard containment standards and other pertinent guidelines and legislation, as developed or amended in the future.

1. Present Research Proposal

a. As the present research proposal is now confined to the use of F. novicida without animal experimentation, using the techniques previously quoted and recognizing that this organism has no known human health impact, the Panel concludes that such a research activity can be safely conducted in the present Level 2 Biohazard facility at the University of Victoria.

b. As the activities in the research proposal use F. novicida only, the Panel concluded that there is no indication for the use of F. tularensis (LVS) vaccine for staff or students.

c. Although the present research is not considered hazardous, the Panel recommends that the Biohazards Committee establish an ongoing disease surveillance program for illness which may occur in individuals who work in the laboratories.

d. As all liquid and solid waste is to be sterilized by autoclaving, and as there are no animal carcasses involved in the present proposal, the Panel concludes that there are no additional requirements needed for waste disposal. However, proper procedures and monitoring of the autoclave function on a regular and ongoing basis is a prerequisite.

e. The safety of the building with regard to any seismic activity was considered by the Panel to have been adequately addressed by the University. Personnel were considered to be well informed on emergency procedures related to earthquakes and in the prevention of hazards. The structure of the research facility conforms to the 1980 building code requirements, and the Panel noted that the staff continue to work to improve the internal security aspect relative to seismic activity and felt that the University should be commended for this effort.

f. The Panel felt that emergency response protocols were adequate for the type of research currently being proposed.

g. It was recommended that the security of the laboratory facilities should be increased by the replacement of all glass panel doors to the biohazard and animal care areas.

h. As no animal experiments are involved in the current research proposal, the Panel is satisfied that there is no threat involved to the surrounding wildlife population.

2. Future Research Considerations

a. Any intention by the University to conduct research on Francisella organisms other than Francisella novicida, with or without animal infection models should be anticipated well in advance. The University has to make certain that appropriate checks and balances are in place and that each specific proposal is analyzed beforehand to assess its health and safety implications and requirements. In this regard, the Panel recognizes that the Biohazards Committee has already begun to consider the issues involved.

b. The Panel recognized that the principal researcher has not had a great deal of experience with the Francisella organism, and has no previous experience with live infection models. As there is a lack of medical expertise familiar with this type of research and the clinical aspects of tularemia at the University, the Panel felt it was incumbent on the University to develop formalized expert support mechanisms for this type of research with outside agencies to ensure the necessary level of expertise is brought to bear on any future research projects involving hazardous organisms.

c. The Panel felt a similar principle of outside consultations should pertain to the issue of protecting the health of surrounding wildlife by calling in experts from such organizations as the Wildlife Branch of the British Columbia Ministry of Environment.

d. As a suggestion, the Panel felt that the existing principle of having the formalized membership of the local health authority on the Biohazards Committee could be extended to include other areas of expertise.

e. The Panel agreed with the opinion of the Laboratory Centre for Disease Control that any research involving strains of F. tularensis or any animal work with mutagenized F. novicida, will require a Level 3 Biohazard facility. It is emphasized that operational guidelines go hand-in-hand with the physical plant requirements to determine the level of containment achieved. The work necessary to achieve a Level 3 Laboratory should be reviewed prior to commencement and following completion.

In the event of an extension of the research which would necessitate the establishment of a Level 3 Facility, a number of specific written operational protocols would be required pertaining to the operation of the facility. Items covered by such protocols should include, but not necessarily be limited to:

(i) The care and transportation of infected animals within the facility.

(ii) The training and monitoring of all maintenance, research students and support staff in procedures to ensure their safety and protection.

(iii) The sterilization, packaging, transportation and disposal of all biohazardous waste.

f. Written waste disposal protocols consistent with all local Provincial and Federal requirements should be developed with the appropriate agencies. Recognizing that unexpected developments can occur with such research, it is incumbent on the Biohazards Committee to be regularly and fully appraised of developments in all phases of the research. This would include any change in the virulence of organisms involved, reports of the ongoing monitoring systems in place, and any unusual incidents which may occur.

g. The Panel is satisfied that in health and safety matters, a formal protocol exists which allows the Biohazards Committee and its staff adequate access to the President of the University to deal with any unusual circumstances or problems.

SUMMARY

The Panel felt very optimistic about the continuing development of appropriate safeguards at the University and that lessons were being learned by all concerned along the way. With a concerted effort to develop a positive working relationship between the University and appropriate outside regulatory and consultative agencies, the Panel is confident that the public health and safety issues can continue to be adequately addressed.

The Biohazards Committee and staff must continue to receive the support of the University and must continue to pay particular attention to written protocol development to make sure that adequate procedures are developed in anticipation of future research proposals if work with virulent strains of organisms and animal models is contemplated.

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